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Morphological, stomatal, pigmentation, and biomolecular characteristics of a few epiphytic orchid species of India

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ABSTRACT

In order to identify suitable species that can be used for cross-breeding and propagation for horticulture purposes four endemic and epiphytic orchid species, *Aerides crispa* Lindl*., A. maculosa* Lindl*., A. ringens* Fischer. and *A. odorata* Lour. were studied by comparing their leaf morphology, stomata, pigmentation, and biomolecules. Standard methods were used for the evaluation. *A. odoratum,* native to the lowest altitude, was characterized by thin, long leaves with the lowest specific leaf area and higher water content. Among the four species studied, stomata were bigger in size and less in number in *A. odoratum.* Maximum total soluble sugars, soluble proteins, amino acids, and starch contents, an indicator of a better photosynthesis rate were noted in *A. odoratum.* Total chlorophyll and carotenoid contents per unit leaf area were higher in *A. odoratum* and *A. ringens*. Our results concluded that variation in environmental temperature, humidity, sunlight intensity, and exposure of orchids at different altitudes may cause alteration in traits that can be inherited over a period.

Key words: *Aerides*, Biomolecules, Morphology, Pigmentation, Stomata.

INTRODUCTION

Orchids are among the largest groups of cosmopolitan flowering plants distributed worldwide, especially in tropical regions (Hartati *et al*., 8). The taxonomy of orchids is characterized by the unique morphology of flowers, like size, shape, colour, durability of flower, number of flower buds, and length of stem (Devadas *et al*., 5). *Aerides*, the Cat's-tail Orchid or the Fox Brush Orchid, is a genus of noble evergreen epiphytic orchids that is outstanding in horticulture for their distichously arranged delightfully curved leathery leaves and their long graceful raceme of scented beautiful flowers (Kocyan *et al*., 12). *Aerides* species are endemic to different parts of India. *Aerides crispum, A. maculosum* and *A. ringens* var. III are found at 1000 m, 1200 m, and 1500 m elevations at Western Ghats. *A. odoratum* is the inhabitant of lowland forests at elevations of 500 m in the Eastern Himalayas (Linthoingambi *et al*., 13). Thicker leaves with lesser water content and lower specific leaf area can characterize the species that grow in intense sunlight. They usually have more total chlorophyll (Chl) and carotenoid contents. The Chl a/b ratio is usually high in these plants compared to the leaves growing in shade (Lichtenthaler *et al*., 13). It is also reported that the leaves exposed to intense sun exhibit higher rates of photosynthetic CO_2 assimilation which is associated with better stomatal conductance (Fanourakis *et al*., 7).

It is evident from many studies that the numbers and size of plants generally decline with the increase in altitude due to a drop in temperature and fertility of the soil, as well as a reduction in precipitation level and the growth season (Timsina *et al*., 20). The morphology of plants is affected by ecological conditions such as light, temperature, humidity, soil, etc., which are directly related to the altitude and elevation they inhabit (Zang *et al*., 21). Stomatal density (number of stomata per unit leaf area) and stomata size are also affected by prevailing climatic conditions such as light intensity, temperature, water availability, and leaf position on the crown (Paul *et al*., 16). Consequently, stomatal density strongly affects the rate of photosynthesis, which will define the production of biomolecules in plants (Timsina *et al*., 21).

All *Aerides* species are epiphytic in nature and grow on other host plants. All *Aerides* species studied grow in shade but at different altitudes. Thus, comparing morphology, pigmentation, biomolecules, and stomatal density of different species of *Aerides* will be a fundamental step to differentiate these species. Although morphological characteristics of *Dendrobium*, *Phalaenopsis*, and *Vanda* spp. have been investigated (De *et al*., 2,3; Deng *et al*., 4), no similar data is available for the *Aerides* species. The present study compared and co-related the morphological, stomatal, pigmentation, and biomolecular characteristics of various *Aerides* species found at different elevations in India. It will help growers identify suitable species that can be used for cross-breeding and propagation for horticulture.

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MATERIALS AND METHODS

Orchid species *A. crispa, A. maculosum,* and *A. ringens* were identified from their natural habitat on Nilgiri hills, Western Ghats, India. *Aerides odoratum* was identified from the mountains of Nagaland. Ten plants of each species were selected for the study. Fresh mature leaves were collected for morphological, stomatal, pigmentation, and biomolecular characterization. Mature leaves were collected and dried at 60 °C to estimate sugars, amino acids, and starch.

In the present study, leaves and flower samples were collected and studied. The morphology of leaves and flowers was observed by the naked eye and measured by a ruler wherever required. The leaf-peel method was used to study the stomatal complex (Eisele *et al*., 6). Microscopic observations and photographs were captured to determine the density of stomata and stomatal index. Stomatal density (SD) was calculated by counting the stomata present per square millimetre. The stomatal index was calculated using the formula: (S/E+S) × 100, where S is the count of stomata present per square millimetre and E is the number of ordinary epidermal cells per square millimetre.

Photosynthetic pigments were extracted from the basal end and tip of the leaves using acetone. The concentration of Chlorophyll a (Chl a), Chlorophyll b (Chl b), total Chlorophyll, and carotenoid were calculated by using Lichtenthaler *et al*. (13) equations respectively. Chl a/ chl b ratio was also determined. Anthocyanin was extracted from flowers using a modified method described by Srivastava (19).

Leaves were initially dried in the oven for 2 hrs and then homogenized in 80% ethanol. The mixture was centrifuged for 10 min at 2000 rpm and the supernatant was decanted. Subsequently, 3 ml of 80% ethanol was added to the residue of the homogenized mixture, and centrifuged again. This extraction process was repeated two times to confirm the full retrieval of sugars and amino acids. The residue was used for the estimation of starch. The supernatant was kept in a boiling water bath to evaporate, and the residue was eluted with ethanol (20%) and used for the estimation of sugars and amino acids. Total sugar and starch contents were measured by using the modified Chow and Landhausser method used by Srivastava (19). The quantity of reducing sugars was estimated as described by the DNSA method. The amino acid contents were estimated by the ninhydrin method as described by Srivastava (19). Data were noted for 10 samples of each species. Mean and SD were calculated using SPSS software. A confidence interval of 95% was taken for statistical analysis.

RESULTS AND DISCUSSION

Orchid species can be identified based on the size of the leaves. Leaf morphology was closely related among all four species *viz*., *Aerides crispum, A. maculosum, A. ringens* and *A. odoratum* but can be statistically distinguished based on the size of the leaf and stem (Table 1). Shape and size of leaves are important factors influencing the absorption of light energy. *A. odoratum,* abundant at lower altitudes is exposed to higher light intensity. Their leaves are strap-shaped, longest (23.43 cm), broadest (3.02 cm), less leathery, and more curved among all the four species. According to Hayes (9), high temperature is a nondirectional environmental stimulus, which might be responsible for the large number of longer leaves and longer stems of *A. odoratum*.

Table 1. Morphological characters of leaves of different *Aerides* species.

Characters	*A. crispa*	*A. maculosum*	*A. ringens*	*A. odoratum*
Length (cm)	15.0±0.6	22.6±1.8	14.86±1.1	23.43±2.7
Breadth (cm)	2.88±0.9	2.26±0.7	1.83±0.67	3.02±1.1
Leaf thickness (mm)	0.5 ±0.06	0.5± 0.03	0.7 ±0.03	0.2 ±0.01
Leaf orientation	Horizontal	60° from stem	Horizontal	Horizontal
Number	12.5± 3.0	7.0 ±2.0	9.3 ±2.5	13.8± 3.2
Fresh weight (mg)	5.17±0.6	4.78±0.45	4.48±0.22	5.10±0.7
Dry weight (mg)	0.81±0.04	0.78±0.23	0.71±0.03	0.79±0.06
Water content (%)	84.33 ±2.4	85.2± 3.1	82.2± 2.9	84.51± 2.7
Leaf mass / area (mg/cm)^2	0.12± 0.02	0.13± 0.01	0.13± 0.02	0.14± 0.03
Specific leaf area (cm^2/mg)	6.45±0.8	6.86±1.3	7.04±1.2	4.80±0.9
Length of stem (cm)	6.04±0.8	5.30 ±0.7	12.75±1.5	6.50±1.2

Epidermal studies showed very few or no stomata on the adaxial surface. It confirms that leaves are hypostomatic. This is in conformity with other orchids like *Ludisia discolor, D. nutantifolium* (Rasmussen and Rasmussen, 18). Stomata present on the tip and base end at the lower epidermis varied among the four species studied. Among the four *Aerides* species, the highest stomatal density (192.33) was found in *A. ringens* tip whereas the lowest density (61.33) was recorded in *A. odoratum* basal end (Fig. 1). Stomatal index ranged from 4.2 to 6.7 at *A. ringens* basal end and *Aerides crispum* tip, respectively (Fig. 2). Stomatal density and stomatal index are a reflection of the plant environment interaction. Stomatal length, width, area, and aperture size were noted maximum in *A. odoratum.* Light intensity, temperature, and humidity are usually positively correlated with stomatal size and stomatal index which lead to better stomatal conductance (Fanourakis *et al*., 7). In general, stomatal density increases from leaf base to tip. Similar observations were made in this study for all *Aerides* species (Table 2). Stomata density and size determine the CO_2 intake of plants for photosynthesis, but only the concentration of CO_2 does not limit photosynthesis (Kardiman and Raebild, 10). In the present study, *A. odoratum* had statistically significantly greater biomolecule contents compared to other species (Table 3). It might be explained as the larger size of stomata on the leaf surface of *A. odoratum* might increase the rate of carbon dioxide uptake and assimilation, and consequently the photosynthesis rate and production of biomolecules (Rasmussen and Rasmussen 18).

All the epiphytic species usually have higher total chlorophyll, lower chlorophyll a:b, and greater carotenoid and anthocyanin content than species of the intense light environment (Parry *et al*., 15). In this study, *A. odoratum* and *A. ringens* had almost the same total chlorophyll, carotenoid, and anthocyanin

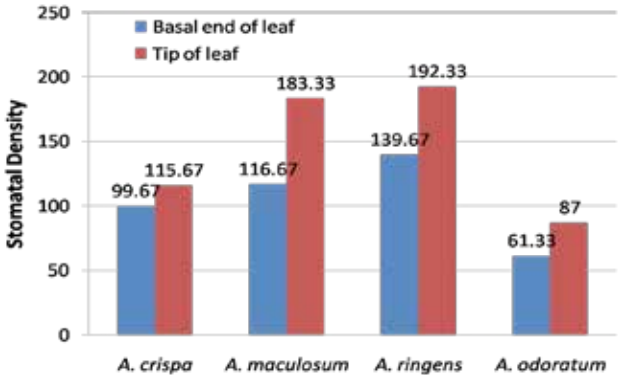

Fig. 1. Stomatal density in the lower epidermis of different orchid species.

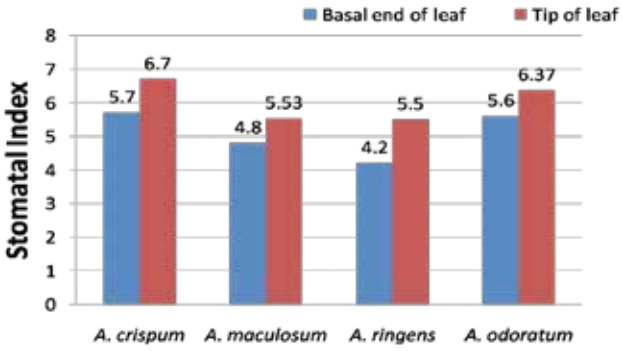

Fig. 2. Stomatal index in the lower epidermis of different orchid species

Table 2. Characterization of stomata in the leaf of *Aerides* species.

Characters	*A. crispa*	*A. maculosum*	*A. ringens*	*A. odoratum*
Stomatal length (µm)	48.32± 1.72	45.81±2.34	40.54±3.13	51.02±2.74
Stomatal width (µm)	36.31 ±1.33	33.91± 1.55	30.13± 2.81	39.66±2.15
Stomatal area (µm^2)	1376.23±15.75	1218.86 ± 21.82	996.36±1 6.74	1585.61± 18.91
Stomatal aperture (µm)	20.48±1.66	18.28±1.96	15.00±1.63	24.7±3.13
Guard cell width (µm)	9.43±0.81	8.51±0.74	7.76±0.92	12.7±2.53

Table 3. Biochemical composition of different species of *Aerides.*

Characters	*A. crispa*	*A. maculosum*	*A. ringens*	*A. odoratum*
Total soluble sugar	128.33±6.7	165.00±7.23	91.00±2.60	172.67±9.80
Reducing sugar	159.33±4.4	196.67±6.17	121.67±9.90	221.66±10.70
Soluble protein	27.52±3.46	16.43±2.91	48.80±3.01	41.75±3.81
Amino acids	75.07±4.96	58.19±5.62	55.62±4.83	85.67±5.62
Starch	85.04±5.70	126.34±9.83	71.78±8.35	142.31±10.32

Table 4. Chlorophyll content in different *Aerides* species.

Characters	*A. crispa*	*A. maculosum*	*A. ringens*	*A. odoratum*
Total Chlorophyll (µg/g FW)	4.64±1.2	4.76 ±0.71	7.06 ±0.91	7.07 ±1.41
Chlorophyll-a/Chlorophyll-b ratio	1.13 ±0.03	1.56 ±0.06	1.92 ±0.07	1.23 ±0.08
Chlorophyll / soluble protein ratio	0.17 ±0.07	0.29 ±0.05	0.14 ±0.07	0.17 ±1.3
Carotenoid (µg/g FW)	0.67±0.02	0.73 ±0.05	1.50 ±0.09	1.42 ±0.13
Anthrocyanin (µg/g FW)	0.30±0.04	0.23±0.03	0.4±0.05	0.38±0.02

content reflecting that pigmentation is not affected by environmental factors (Table 4). Greater carotenoid and anthocyanin content may protect chlorophyll from photooxidation and ultraviolet radiation. This result can be concluded as the leaf chlorophyll contents and pigments are a proxy for photosynthesis, but many other factors are also involved in this process (Croft *et al*., 1).

AUTHORS' CONTRIBUTION

Conducting experiments and writing this research (BSJ and DS), Conceptualization of research and editing of the original draft (DS).

DECLARATION

There is no conflict of interest among authors.

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